THE STATE-OF-THE-ART CHEMICAL ANALYTICAL METHOD FOR DETECTION OF SODIUM AZIDE BY ^{14}N NMR SPECTROSCOPY

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Accepted August 19, 2015

Abstract

Sodium azide is acute poison similar to cyanide. Due to its attractive chemical and physical properties it is widely used in many spheres including automotive industry, medicine, pharmaco-chemistry, agriculture and even everyday life. Detection of sodium azide becomes more demanding nowadays than several decades ago. We propose to use of ^{14}N NMR spectroscopy to detect and quantify sodium azide in aqueous solutions and extrapolate calibration results for real time detection of unknown concentrations. The results of this methodology relying in measurement of 1D ^{14}N NMR spectra at the lowest concentration of sodium azide aqueous solutions.

Introduction

Sodium azide is acute poison similar to cyanide. Sodium azide is in the group of alkali metal azides, it is water soluble and currently used in many applications, from industry to everyday life.

The Organization for Economic Co-operation and Development (OCDE) has included sodium azide in the list of 5,235 High Production Volume Chemicals (HPV) with a production or import greater than 1,000 tons per year [1].

One particular important area of use of sodium azide is in the agricultural sector. Sodium azide is among the great variety of chemicals which are frequently used as fermentation inhibition in wine production.

Since 2000s sodium azide started to be used as pesticide, herbicide and insecticide [2]. In one study during eight year period usage of sodium azide in soil reached the impressive value of 336 kg / ha [3].

In 2014, International Organization of Vine and Wine (OIV) released the revised "Compendium of International Methods of Analysis" for the detection of preservatives and fermentation inhibitors. They proposed two methods of detection sodium azide which are HPLC and colorimetric method. This methods are relative obsolete despite the fact that it was modified in 2006, based on originally developed works Swaring & Waldo [4] and Battaglia & Mitiska [5]. The colorimetric method proposed is also very obsolete and neither specific nor sensitive. While, in recent years was discovered possibility of reaction based detection of sodium azide at small quantities as 21 ppb [6].

In parallel, concern was raised by environmentalist and atmospheric scientists about the safety of the use of sodium azide. Despite the widespread opinion of sodium azide proponents for its use in water and soil, arguing that this chemical undergoes rapid hydrolysis and degradation (Rodrigues–Kabana et al.) [7 – 10], their opponents (Betterton et al.) [11 – 13] claimed that it is not exactly what it can be anticipated, since they discovered water and soil samples containing residual amounts of sodium azide.

In the recommendations for disposal of sodium azide in industry or workplace, the standard method for its neutralization is manifold dissolution in water and release in sewage by the Royal Society of Chemistry [14], at the same time, American Azide Corporation strictly require a chemical reaction for the inactivation of wastes of sodium azide before draining [15].

In this context, it is clear that the detection of sodium azide becomes more demanding nowadays than several decades ago.

We propose to use of ^{14}N NMR spectroscopy to detect and quantify sodium azide in aqueous solutions and extrapolate calibration results for real time detection of unknown concentrations. The results of this methodology relying in measurement of 1D ^{14}N NMR spectra at the lowest concentration of sodium azide aqueous solutions.

Aims and objective

The aim of the study is to apply ^{14}N NMR spectroscopy to detect ^{14}N spectra of the sodium azide at several concentrations to determine minimum detection limit.

We followed to fulfill the following objectives:

1) We studied the detection limit of NaN_3 with ^{14}N NMR. The minimum concentration of NaN_3 that can be measured with ^{14}N NMR in the spectrometer in a standardized and reasonable amount of time.
2) We determined the concentration of any unknown sample of NaN_3 prepared under analogue conditions in water, as far as it is equal or greater than the ^{14}N NMR detection limit of NaN_3.
3) The next is to perform calibration of NaN_3 samples with dilution experiments and calculate calibration curve for ^{14}N NMR sensitivity for detection of sodium azide of unknown concentration.

To estimate the minimum detectable concentration of sodium-azide, we need to prepare a sample with known concentration. To acquire the spectrum under some standardized conditions (in particular the total measurement time) and repeat the spectrum 5 or more times diluting the sample.

Then we extrapolated the calibration curve of NMR sensitivity (signal-over-noise ratio S/N) with the respect to NaN_3 concentration to determine the minimum required concentration, which is detectable in the spectrometer.

Materials and Methods

The experimental part of this project is performed in the Magnetic Resonance Unit at the Center of Technology Innovation and Transfer (CACTUS) of the University of Santiago de Compostela. Experiments were conducted during 2012–2014 and obtained results analyzed.

University of Santiago de Compostela (USC) is equipped with NMR spectroscopy and propriety technology of MESTRE Labs, which is the software used worldwide.

The Magnetic Resonance Unit at the University of Santiago de Compostela, provides the optimum research instrumentation required for this part of the project. The NMR facility provides three state-of-the-art high magnetic field NMR spectrometers of 500 and 750 MHz.

Experimental

Sodium azide and deuterated water (D_2O) were obtained from Sigma-Aldrich. Azide standards were prepared using sodium azide in D_2O, in which target concentrations were set relative to free azide concentration. Test samples were prepared at descending target concentration in D20. Stock sample of 100 mM concentration solution of sodium azide 0.5 ml was transferred into an NMR tube. The sample NMR tube is placed into a magnetic field. A radio frequency pulse is then sent through the sample solution in order to orient the magnetic moments of the nuclei in the solution. As the magnetic moments relax, they exhibit free induction decay (FID). The free induction decay is Fourier transformed into a NMR spectrum. The NMR spectrum displays chemical shifts for the individual nuclei of nitrogen; and from these chemical shifts, the structure of the compound was determined.

Standard and test sample preparation

1) Preparation of reference standard sample with a compound, which ^{14}N NMR signal is used as the reference of concentration. This sample is important because it allows measuring the unknown concentration of NaN3 in any sample that we prepare in the future.
3) Reference compound: 100 % CH_3NO_2 total volume of 700 microliters.

Reference compound 100 % Nitromethane (CH_3NO_2) 600 microliters was placed in a standard NMR tube and the remaining 100 microliters in a special capillary. The capillary with the reference compound is inserted inside of standard NMR tube. We prepared the initial sample of NaN3 in solution in a "narrow wall" NMR tube.

The starting concentration of NaN_3 is 100 mM in this sample. Then the capillary is inserted with the reference sample inside this standard NMR tube. We measure the 14N spectrum of the sample by 4 or 5 dilutions of the original NaN_3 concentration using dilution 5 times.

Results:

A preliminary test was carried out in our lab for the measurement of the 1D ^{14}N NMR spectrum of sodium azide in water solution. The spectrum was obtained with very good quality in only 30 s of measurement time. The sample 100 mM gave the expected three peaks in ^{14}N NMR (two peaks for NaN_3 and one peak for CH_3NO_2). The assignment of the peaks of the 1D ^{14}N spectrum of sodium azide in water is provided in **Figure 1**.

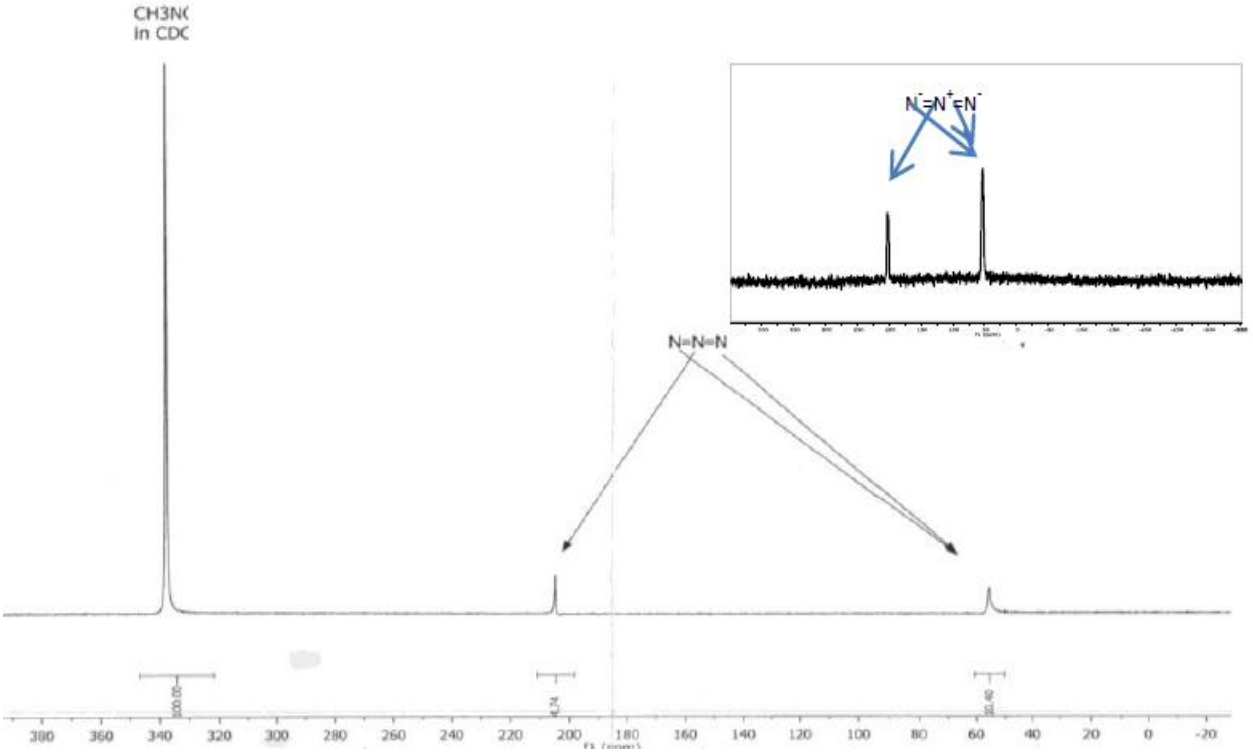


Figure 1. ^{14}N NMR spectrum of 100 % CH_3NO_2 and 100 mM Sodium azide (NaN_3 in D_2O).

The chemical shifts obtained are in excellent concordance with those described for this molecule in the NMR spectral databases, and also in concordance with those expected for its structure. The 1D ^{14}N NMR spectrum of sodium azide shows just two peaks corresponding to the two types of nitrogen atoms of this molecule. The two external nitrogen atoms resonate at ~ 50 ppm, and the central nitrogen atom at ~ 200 ppm. As expected, the former peak has double intensity than the later one since it corresponds to two nitrogen atoms.

The ^{14}N chemical shifts resonances are spread out in an extremely broad range that covers 900 ppm. Such dispersion is favorable for our purposes of detecting sodium azide in mixtures, since it reduces the chances of accidental signal overlapping with the ^{14}N signals of other nitrogenated compounds. As the ^{14}N-spectrum of sodium azide has two peaks, and the

same happens with its ^{15}N-spectrum. In both cases the peaks are easily to interpret; the less intense one correspond to the central N atom, and the double intense one corresponds to the two external nitrogen atoms.

1D ^{14}N NMR spectrum of sodium azide 100 mM in H_2O obtained at 300 K in a Bruker Avance I NMR 11.7 T spectrometer. The spectrum was obtained in 30 s with 64 scans. The assignment of the peaks to the azide molecule and $CH3NO_2$ are shown of the **Figure 2** (Magnetic Resonant Unit, CACTUS, University of Santiago de Compostela).

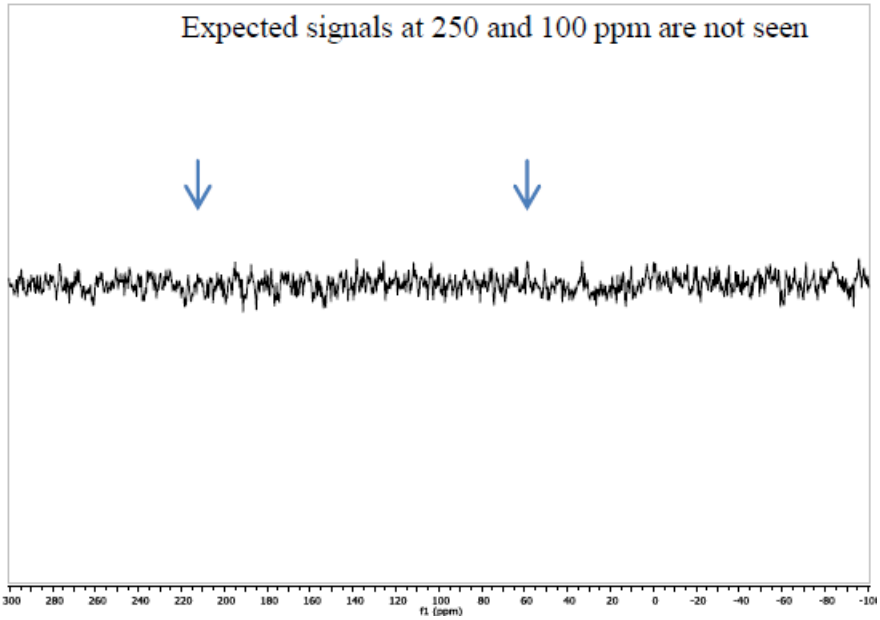


Figure 2. ^{15}N NMR spectra of 100 mM sodium azide.

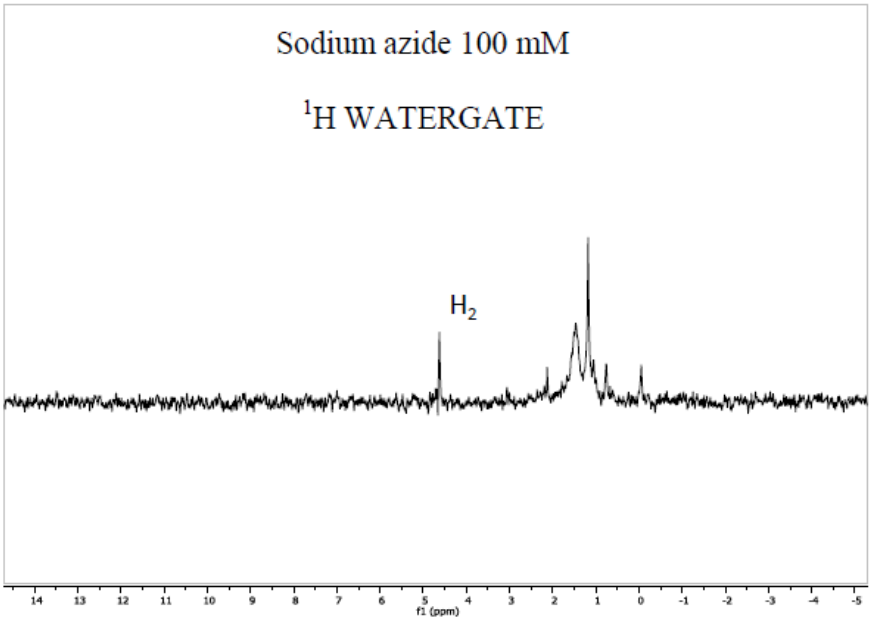


Figure 3. Proton ^{1}H NMR spectroscopy of NaN_3.

The ^{15}N peaks of sodium azide are not visible because they are below the noise level (S/N). To observe them, it is sure that a longer measurement time is needed; it means to use many more scans to average the noise to a lower level, or alternatively, to increase the

concentration of sodium azide in the sample so as to get a higher amount of signal per scan. With that sample of sodium azide, it would probably require at least 12 hours of measurement time (or even more) to make the expected ^{15}N peaks appear over the noise level.

The ^{14}N-spectrum of NaN_3 is highly sensitive, much better than the equivalent experiments with ^{15}N in the same molecule. The measurement of the 1D ^{1}H NMR spectrum of sodium azide solutions in water is not practical. Depending of pH, protonated forms of sodium azide could be formed and in principle detected with proton NMR, however those forms do not lead to distinguishable peaks in the ^{1}H spectrum because they overlap with the strong water solvent peak due to their undergoing fast exchange equilibrium with the water. (**Figure 3**).

Under the conditions of measurement, the ^{14}N spectrum of sodium azide had a considerably much better sensitivity than the alternative ^{15}N NMR. Although the concentration of sodium azide in this test sample was considerably high (100 mM), it would be possible to adjust the experimental conditions for the detection of lower concentrations of sodium azide (e.g. 1 mM) within still a reasonable NMR measurement time (e.g. from 30 min. to a few hours). For the next sample we diluted the 100 mM sample by a factor of 4 (concentration of 25 mM of NaN_3) and repeat the spectrum.

To estimate the minimum detectable concentration of the water solution of sodium-azide we need to prepare a sample with known concentrations. To acquire the spectrum under some standardized conditions (in particular the total measurement time) and repeat the spectrum 4 or 5 times diluting the sample. We obtained ^{14}N NMR spectra of 5 different concentrations of NaN_3 water solution, reducing the concentration by factor of 2 each time (**Figure 4**). ^{14}N peaks are observed at 4 mM. Calibration curve was calculated using least squares linear regression method. As an external reference standard was used nitromethane 100 %.

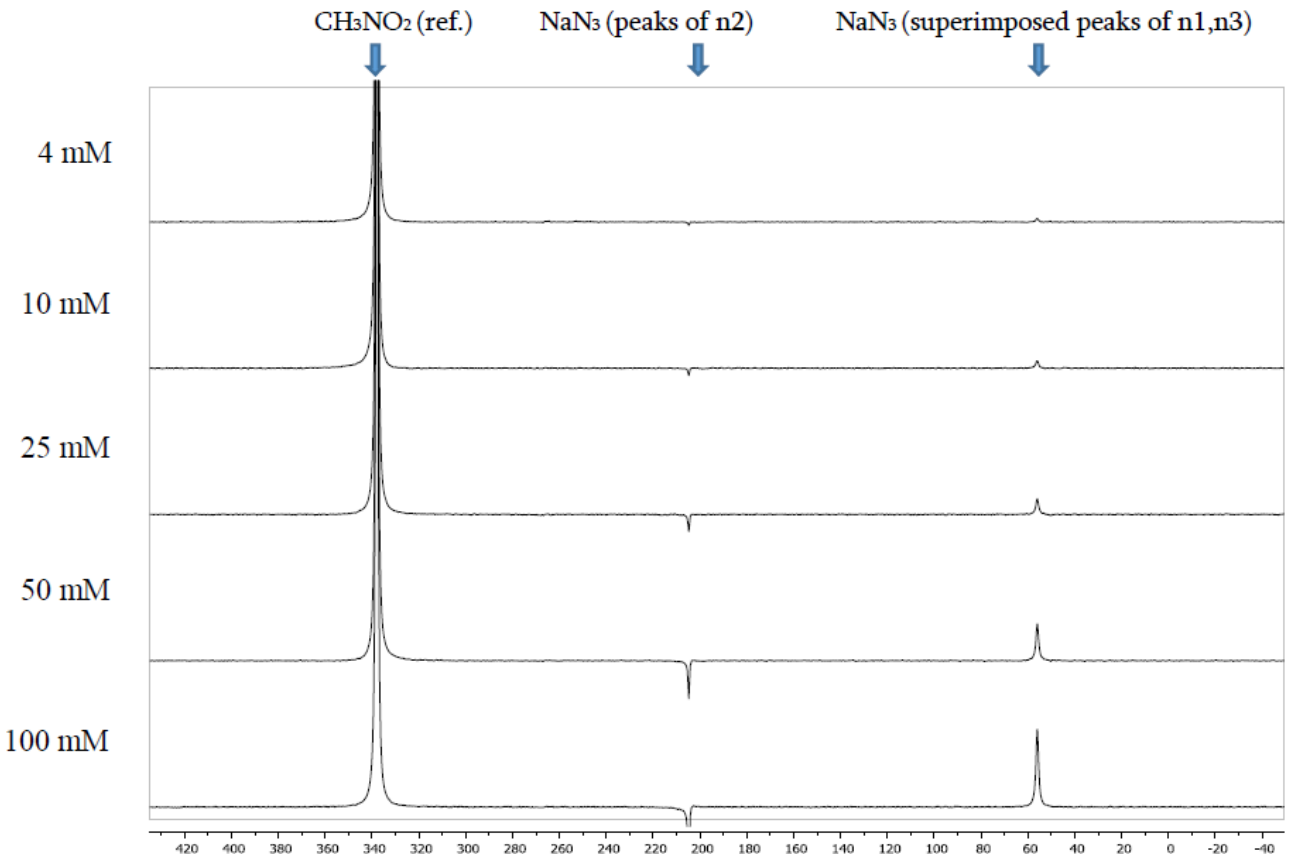
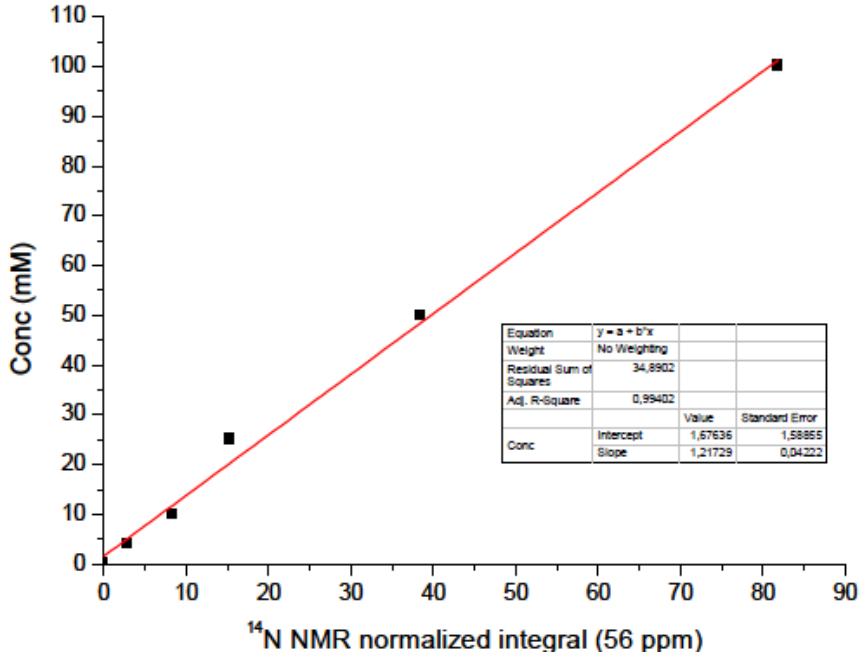
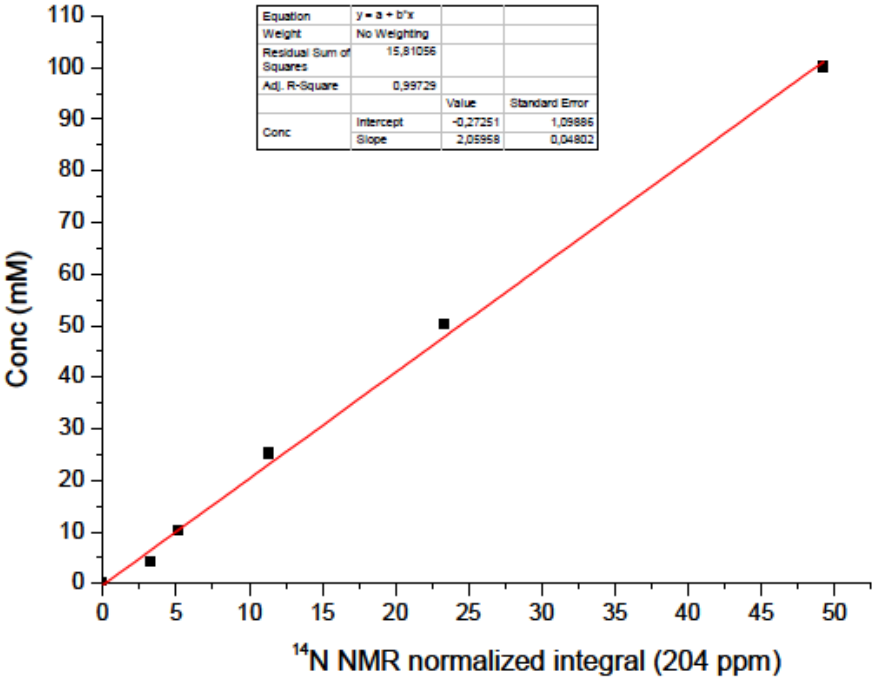


Figure 4. ^{14}N NMR spectra of different concentrations of NaN_3 water solution.

Calibration curve allows using this method for calculation even lower concentration of NaN_3 indirectly. Particularly, we propose to lyophilize analyte sample by following protocol: ^{14}N NMR can be measured with lower concentrations by following standard protocol. As the amount of sodium azide in the sample is only 0.5 mL for NMR essay, we recommend concentrating the analyte sample by liophilization of the water, to get a higher effective concentration of sodium azide for the NMR measurement.



(a)



(b)

Figure 5. Calibration curves (a) and (b).

Liophilization requires freezing under high vacuum a given amount of the analyte sample. The water will sublimate during the liophilization process while the organic molecules and likely sodium azide will remain in the residual frozen solid. After liophilization to resuspend the residual solid in water, and then perform the ^{14}N NMR analysis at 25 °C. For testing of proposed method of liophilization for detection of sodium azide is needed preparation of the sample at a known concentration and measurement by ^{14}N NMR. We evaporate 5 ml of analyte solution and then resuspend up to 0.5 ml we will be able to detect 0.4 mM concentration of NaN_3 in solution using obtained by us calibration curve standard (**Figure 5**) greater by factor of 10 compared to LOD obtained in our experiment.

Discussion

Liquid Nuclear Magnetic Resonance (NMR) spectroscopy is nowadays a well-established, widespread and recognized analytical technique for the identification and quantification of molecules in a mixture. The technique is available in many recognized centers of research and development excellence. Despite the relative insensitivity of NMR compared to other analytical techniques such as Mass Spectrometry (MS), it has the unique capacity of being sensitive to the finest and subtle details of the structure of each molecule in the sample. In particular, the NMR spectrum is sensitive both to the three-dimensional structure of a molecule in solution and to the topology of each one of its covalent bonds.

Moreover, NMR is amenable for detection and quantitative analysis of specific molecules in a mixture without requiring any previous step of separation.

NMR sample preparation is simple and only requires a volume of 0.5 mL. It is also very versatile regarding the type of solvents and temperature that can be chosen. The NMR sample is studied directly under the relevant experimental conditions (e.g. in water solvent, at room temperature, etc.).

The only caution specific for the preparation of NMR samples containing relative concentrated solutions of sodium azide in water, is to handle them with care in a chemical hood and immediately seal the NMR tube after preparation since it changes rapidly to a toxic gas with a pungent (sharp) odor.

A better alternative to detect sodium azide with NMR is to rely in other nuclei such as ^{14}N NMR or ^{15}N NMR spectroscopy. From these two isotopes, ^{14}N is the most abundant in nature (the natural abundance of ^{14}N and ^{15}N is 99.63 and 0.37 %, respectively) and in favorable case molecules, the higher natural abundance of ^{14}N positively correlates with a remarkable higher experimental sensitivity in NMR than ^{15}N.

Nevertheless, such favorable situation of ^{14}N is not always the case; one aspect to consider is that ^{14}N is a quadrupolar nucleus (spin $I = 1$) while ^{15}N has spin ½.

The NMR sensitivity of quadrupolar nuclei is strongly affected not only by its isotopic abundance, but also by its characteristic transversal relaxation rate (R_2). The relaxation rate R_2 affects the appearance of the peak that is generated in the NMR spectrum (peak height and broadening depend on R_2).

According to NMR theory, the expected trend is that ^{14}N nucleus placed in an asymmetric (molecular) environment usually experiences a very efficient transversal relaxation

rate (a high value of R_2), leading to a reduction of the peak height and enhanced line-broadening that severely degrades its NMR sensitivity. The situation is the opposite for ^{14}N nuclei in symmetric (molecular) environments.

Conclusions:

Considering together the large signal dispersion of ^{14}N NMR resonances and the good sensitivity obtained in our preliminary ^{14}N NMR study of sodium azide in water solution, we propose to use of ^{14}N NMR spectra to detect and quantify sodium azide in real case samples e.g., contaminated water or soil extracts, wine, milk, biological fluids, e.g. urine and blood.

The methodology relying in measurement of 1D ^{14}N NMR spectra at the lowest concentration compared to methods described previously with other analytical techniques.

The method has significance to apply in the fields of medicine, pharmaco-chemistry, toxicology, which should open the way for the various possible applications, including neurodegenerative disorders and ageing.

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